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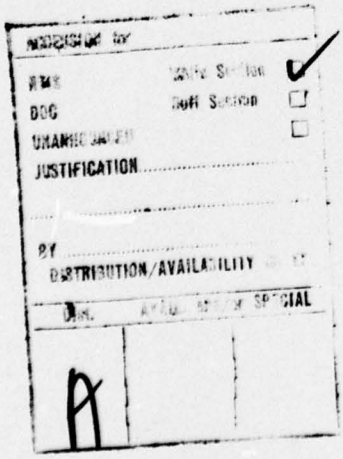

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*Author presenting paper
†Read by title

Parathyroid Hormone as a Possible Factor in Osteopetrosis. W. R. COTTON,* G. A. WILLIAMS, G. K. HARGIS and J. F. GAINES. Naval Dental Research Institute, Great Lakes, IL and VA West Side Hospital and University of Illinois College of Medicine, Chicago, IL

Parathyroid dysfunction has been postulated as a factor in osteopetrosis, usually described as an osteosclerotic condition in which bone resorption fails to keep pace with bone deposition. Hypoparathyroidism could prevent bone resorption. Hyperparathyroidism could (1) directly stimulate osteogenic activity, (2) cause compensatory increase in calcitonin which would inhibit bone resorption, or (3) result secondarily from hypercalcitonism and contribute to osteopetrosis. Until an assay for serum parathyroid hormone (PTH) levels in rats was made available (Hargis et al., Endocrinology 94:1644, 1974), there was no means of directly testing these hypotheses. The availability of a rat (*tl*) model with persistent osteopetrosis (Cotton and Gaines, Proc. Soc. Exp. Biol. Med. 146:554, 1974) further simplified the task.

Serum PTH levels were determined by radioimmunoassay in 5 *tl* rats and compared to serum levels in 6 phenotypically normal littermates (LM) and 7 non-littermates (C).

The mean $\pm$ SD serum PTH concentration (pg/ml) in the 3 groups was: *tl* = 7.91 $\pm$ 0.83, LM = 7.18 $\pm$ 0.69, C = 7.55 $\pm$ 0.95. The mean concentrations did not differ significantly by Student's t-test ($p > 0.05$). These findings are consistent with the previously reported (Cotton and Gaines, Proc. Soc. Exp. Biol. Med. 146:554, 1974) lack of differences in serum Ca and P levels between 3 similar groups of rats. The results suggest that osteopetrosis and its manifestations, at least in the *tl* rat, are not due to parathyroid dysfunction.

Composition and Structure of Streptococcal Glucans as Revealed by ^{13}C Nuclear Magnetic Resonance. B. L. LAMBERTS* and I. C. P. SMITH, Naval Dental Research Institute, Great Lakes, Illinois, and National Research Council, Ottawa, Canada

The wide range of ^{13}C chemical shifts that attends ^{13}C nuclear magnetic resonance (^{13}C NMR) analyses of carbohydrates provides a powerful means of compositional and structural elucidation of complex polysaccharides. The objective of this study was to explore the applicability of ^{13}C NMR to assess the structural characteristics of extracellular glucans from Streptococcus mutans. Water-soluble and water-insoluble glucan fractions were prepared by enzymatic synthesis from strains E-49, Ingbritt, GS-5, OMZ 176, K-1R, and SL-1. The ^{13}C NMR spectra were obtained on the glucans in deuterium oxide under mild reducing conditions at PD 14, using field strengths of 25 MHz and, in some cases, of 68 MHz. The spectra of the glucans from the various strains showed a remarkable similarity. The spectra at 25 MHz indicated two principal types of linkage of the constituent glucosyl moieties (α1→3 and α1→6) whose relative proportions depended upon the strain. The more soluble the glucan, the greater was the proportion of α1→6 linkages. The greater dispersion at 68 MHz resulted in resolution of resonances due to α1→3 backbone and branched moieties. The percent of branched α1→3 linkages was in agreement with the results of standard methylation analyses. The ^{13}C NMR technique provides a means for structural determination that is more rapid and convenient than conventional chemical approaches.

(Supported in part by NMRDC project MR000.01.01.0012).

†Paradontopathic Potential of S. mutans and A. viscosus in the Rat. E. P. LEONARD,* T. A. LABARBERA and I. L. SHKLAIR. Naval Dental Research Institute, Great Lakes, Illinois

The purpose of this study was to determine if the presence of certain bacterial flora would influence the histopathology of the periodontium in rats that had their normal microbiota suppressed with polycillin. Thirty-nine weanling Osborne-Mendel rats were divided into 3 equal groups, by inoculation with either streptomycin-resistant Streptococcus mutans 62-D or streptomycin-resistant Actinomyces viscosus strain T-6, or maintained as non-infected controls. All animals received diet 2000 containing 56% sucrose and distilled water ad libitum. The normal Gram-positive flora was depressed in the weanlings and their dams by incorporating 4 mg/ml polycillin into the drinking water prior to inoculation. Implantations were verified by culture. The animals were killed sequentially at periods of exposure from 20 to 167 days. Decalcified specimens were fixed and prepared for histologic and histochemical evaluation. Commencing at one month a moderate inflammatory infiltrate was observed in all groups. Massive accumulations of apical cementum was a prominent feature at later ages but pocket formation, tissue disruption and bone loss were not observed. A comparison of the inoculated and control animals revealed no notable differences in the severity of tissue changes, location of the epithelial attachment or osteoclastic activity. Under present conditions the bacteria tested do not induce destructive changes in the periodontium of the relatively germ-free rat. Supported by NMR&DC Project No. MR041.20.02 0408A3II.

Bicarbonate-Induced Changes in Sucrose-Metabolizing Enzymes and Cellular Morphology of S. mutans. R. M. OSBORNE,* B. L. LAMBERTS, K. J. BUCK and I. L. SHKLAIR. Naval Dental Research Institute, Great Lakes, Illinois

Previous studies in this laboratory have revealed some effects of culturing S. mutans, strain SL-1, in a chemically-defined medium containing various levels of bicarbonate. The purpose of the current study was to determine if bicarbonate imparted changes in the metabolism of other strains of S. mutans.

The organisms were cultured anaerobically in a gas incubator or fermentor and tested for purity. Strains HS-6, OMZ-61, FA-1, BHT, GS-5, NCTC 10449, SL-1, K1R, AT-10, and P-4 were grown with bicarbonate levels ranging from 0.0% to 1.5% and cellular morphology monitored by Gram-staining or scanning electron microscopy. Strains AT-10, BHT, GS-5, and K1R were cultured at 0.1% and 1.5% bicarbonate levels and extracellular protein isolated. The isolated proteins were then subjected to acrylamide-gel electrophoresis. Sites of sucrose-metabolizing enzyme activity were determined by incubating gels in sucrose solutions and observing polysaccharide synthesis or by assaying for release of reducing sugars.

Increased levels of bicarbonate in the medium caused morphological changes in some strains of S. mutans. Strains NCTC 10449, GS-5, P-4 and AT-10 appeared to be particularly affected in this regard. Increased levels of bicarbonate also caused changes in electrophoretic patterns of enzymes in some cases. No positive correlation was observed between the bicarbonate effect on cellular morphology and the bicarbonate effect on sucrose-metabolizing enzymes.

Supported by NMRDC Project No. MR041.20.02 6048B311.

Cell-Mediated Immunity to Teeth Transplanted Among Genetically Matched Monkeys. G. RIVIERE*, UCLA, J. E. YEAGER and J. DERKOWSKI, Naval Dental Research Institute, Great Lakes, IL

Primates inherit the ability to distinguish self from non-self. Tissues are usually rejected when transplanted between animals who differ as regards these histocompatibility genes. However, experiments employing inbred strains of mice (Klein J, Transplantation 12:500, 1971; Riviere G & Hildemann WH, Transplantation 16:655, 1973) demonstrated that teeth will survive in a weakly disparate host. It is not known if tissue typing will improve the success of tooth transplantation in primates. 120 Rhesus monkeys were typed for RhL-A histocompatibility antigens and 30 animals were paired with partners who shared, or were dissimilar for defined antigens. Unerupted bicuspids were transplanted to orthotopic sites between partners. From the fifth through the 23rd postoperative day, on every other day, recipient peripherial blood lymphocytes were tested for their ability to effect the release of Cr51 from labeled donor lymphocytes. Autologous release was subtracted from allogeneic release, the difference divided by the net total activity in the labeled donor cells and the result was expressed as a %. Pretest evaluations failed to demonstrate donor-specific immunity in any combination. The immunity elicited by single first locus differences were stronger than single second locus disparities. Four combinations of RhL-A identical monkeys proved to be anergic. These pairs are likely to be identical for the other major histocompatibility system (MLR) as well as for RhL-A antigens. These results suggest that teeth may be successfully transplanted among properly matched non-human primates.

Supported by NMR&DC 62711N.51.524.012.010.1.74.

Evaluation of a Selective Medium-Color Test for Streptococcus mutans.
I. L. SHKLAIR* and R. WALTER. Naval Dental Research Institute, Great Lakes, Illinois

This report is part of a study to determine if the presence of Strep. mutans on non-carious tooth surfaces can be correlated with the future development of carious lesions at these sites. A total of 4110 plaque samples were collected from individual posterior interproximal, occlusal and bucco-lingual surfaces of 77 naval recruits with a history of being caries-free (CF) and from 22 caries-active recruits (CA). Each plaque sample was placed in a vial containing 3 ml of a selective medium for S. mutans. The medium consisted of thioglycollate broth w/o carbohydrate 2.4%, lactoalbumin 0.25%, mannitol 0.5%, thallium acetate 0.025%, crystal violet 0.0001%, and brom cresol purple as the indicator. A color change in the medium from purple to yellow was presumptive evidence for the presence of S. mutans. To confirm the presence or absence of S. mutans, the samples were plated on mitis-salivarius agar when a color change was noted, or after 5 days if there was no color change. The rate of color change was usually dependent on the number of S. mutans present in the plaque sample. Approximately 50% of the samples changed the indicator in the vials in 2 or 3 days, whereas, 7% changed color at day 1 or in 4-5 days. The CF group had 65% of the sites sampled positive for S. mutans compared to 86% in the CA recruits. The color change, based on the presence or absence of S. mutans, was valid in 93.7% of the tests. False positives amounted to 5.8% and false negatives 0.45%. The color test can be useful in large scale epidemiological determinations for the presence or absence of S. mutans.

Supported by NMRDC Project No. MR041.20.

Site Distribution of Streptococcus mutans in Caries-Free and Caries-Active Recruits. R. WALTER* and I. L. SHKLAIR. Naval Dental Research Institute, Great Lakes, Illinois

This investigation attempted to define, semi-quantitatively, the extent and site distribution of S. mutans infection in 77 caries-free (CF) and 22 caries-active (CA) recruits. Plaque samples were obtained from approximately 36 separate sites in each subject including: (1) the occlusal (OC) surfaces of all molars and premolars, (2) the interproximal (IP) spaces of all posterior teeth and (3) buccal and lingual (BL) samples from each quadrant. Samples were placed into a colorimetric test medium. For each of the three anatomical areas the data were grouped according to whether the samples became positive after one day, two or three days, four or five days or remained negative after five days. In CF recruits, S. mutans was isolated from 73.2% of IP sites, 51.4% OC sites, and 68.1% BL sites compared to 96.6%, 72.3%, and 95.9% in the CA group, respectively. CF versus CA comparisons were statistically significant at each anatomical site ($p < .01$). Within CF and CA groups, the frequency of positive isolations at IP and BL sites was significantly higher than for OC sites ($p < 0.05$). When the positive samples were grouped according to the 3 anatomical sites, χ^2 analysis indicated a statistically significant more rapid color change in the CA group than in the CF group ($p \leq .05$). Overall this investigation indicated that the CA recruit was more completely and more heavily infected with S. mutans than his CF counterpart.

Supported by NMRDC Project No. MR041.20.02 6049A3IJ.

Gingivitis, Bacterial Plaque, and Streptococcus mutans in Naval Recruits from Saudi Arabia. M. R. WIRTHLIN*, H. J. KEENE, and I. L. SHKLAIR, Naval Dental Research Institute, Great Lakes, IL

The purpose was to test the hypothesis that Strep. mutans infections of bacterial plaque are independent of gingival inflammation. A complete oral examination was performed on each of 52 Royal Saudi Naval Force recruits, 17 to 26 years of age, before any treatment. A clinical diagnosis of the periodontal status was determined, and then the Navy Periodontal Screening Examination which consists of the Navy Periodontal Disease Index (NPDI) and the Navy Plaque Index (NPI). Supragingival plaque was collected from occlusal, proximal, and buccolingual surfaces, pooled, and grown on mitis-salivarius agar. The percentage of S. mutans to total streptococci was determined. Mean values for DMFT components were: DT=3.3; MT=0.3; FT=0.1; and DMFT=3.5±2.9 S.D. Mean values for NPDI and NPI Total scores increased as the clinical diagnosis became more severe: localized chronic papillary gingivitis: NPDI=11, NPI=78; loc. chr. marginal ging.: NPDI=15, NPI=92; generalized chr. pap. ging.: NPDI=16, NPI=93; gen. chr. marg. ging.: NPDI=20, NPI=97; ging. with loc. periodontitis: NPDI=33; NPI=98. Correlation analysis indicated a moderately strong relationship between NPDI and NPI ($r=0.493$, $p=.001$), and a stronger relationship between the gingival portion of the NPDI and NPI ($r=0.806$, $p=.001$). The mean percentage of S. mutans to the total streptococci was 6.0±8.2 S.D.; and had low correlations to NPDI ($r=0.033$, $p>.05$), to NPI ($r=-0.015$, $p>.05$), and to the gingival portion of the NPDI ($r=0.116$, $p>.05$). Young men from Saudi Arabia had low caries prevalence and high gingivitis levels. The prevalence of S. mutans in bacterial plaque had no strong relationship to gingivitis.

Supported by NMR&DC Project No. MF12.524.012 000AG31.

Tooth Transplantation in RhL-A Typed Monkeys. J. E. YEAGER,* G. R. RIVIERE and G. W. DILS. Naval Dental Research Institute, Great Lakes, Illinois

The purpose of this study is to test the hypothesis that genetic typing followed by histocompatibility matching will enhance tooth allograft survival. Thirty Rhesus monkeys ranging in dental age from 25 to 65 months were selected for this study and were designated as experimental pairs on the basis of their RhL-A antigens. Thirty maxillary bicuspid allografts and thirty mandibular bicuspid autografts were performed. All transplanted teeth that had erupted at the time of surgery were placed in erupted positions. All unerupted donor teeth were positioned completely within alveolar bone of recipient and covered with a muco-periosteal flap. Besides periodic immunologic monitoring consisting of cross match-type cytotoxicity experiments, clinical and radiographic examinations were scheduled for the following postoperative intervals: for every 2 weeks during the first 8 weeks; for every 4 weeks during the next 32 weeks; for an 8 week interval from the 40th to the 48th weeks; and for every 12 weeks during the remaining 60 weeks of the 108 week study. The most recent postoperative examinations ranged from 8 weeks for the last pair of animals operated to 32 weeks for the first pair operated. Two allografts and one autograft have been lost. Of the fifty-seven remaining teeth, only one shows gross root resorption. There has been no tooth loss and no evidence of resorption in the case of the six allografts in which the donor and recipient animals were perfectly matched in regard to RhL-A antigens.

Supported by NMR&DC Project No. MR041.20.

Microbial Aspects of Dental Caries: Proceedings of the St. Simons Island Workshop, June 21-24, 1976

1. SHKLAIR*, I. L. and KEENE, H. J. - "Biochemical Characterization and Distribution of Streptococcus mutans in Three Diverse Populations."

*Author presenting paper

Biochemical Characterization and Distribution of *Streptococcus mutans* in Three Diverse Populations

Streptococcus mutans was separated into 5 biotypes on the basis of 6 biochemical tests. The biotypes correlated with their genetic and serologic groupings. The biotype identification was based on the fermentation of mannitol, with and without 2 units/ml of bacitracin, sorbitol, raffinose, melibiose, and the production of ammonia from arginine. Three diverse populations, U. S. naval recruits, Saudi Arabian naval personnel, and Hawaiian children were examined for the prevalence and biotypes of *Strep. mutans* carried. *Strep. mutans* was found in 90 percent of the plaque samples. The Saudi Arabians had significantly higher multiple biotypes as well as a higher percentage of biotypes IV and V than the U. S. recruits or Hawaiian children. Biotype I was the most frequent biotype isolated in all 3 populations.

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Rhesus monkeys						
Streptococcal glucans						
Streptococcus mutans						
Tooth transplantation						